



Effects of Aqueous Leaf Extract of *Chromolaena odorata* on Petrol-Induced Bronchiectasis in Adult Wistar Rats

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Abstract

BACKGROUND AND AIM: The most common cause of lung diseases among people with petrol-related occupations is petrol poisoning which has significant morbidity and mortality if left untreated. This study aimed to investigate the effects of aqueous leaf extract of *Chromolaena odorata* on petrol-induced bronchiectasis in the adult Wistar rats.

METHODOLOGY: Thirty adult Wistar rats weighing between 240 g and 270 g were divided into five (5) groups of six (6) rats per group. Animals in group A were placed on feed and water only. Animals in group B were exposed to 100 ml of petrol vapor via inhalation. Animals in group C received 500mg/kg body weight per day of *Chromolaena odorata*. Animals in group D were exposed to 100 ml of petrol vapor via inhalation and received 250mg/kg body weight per day of *Chromolaena odorata*. Animals in group E were exposed to 100 ml of petrol vapor via inhalation and received 500mg/kg body weight per day of *Chromolaena odorata*. The extract was administered for 30 consecutive days via an orogastric tube.

RESULTS: Groups A, C, D and E revealed normal histoarchitecture of the lung; normal alveoli, patent bronchiolar lumen and normal arteries. There were observable histological variations in the lung tissues of Group B rats which include bronchiolar destruction and widening, severe bronchiolar mucosal ulceration and vascular ulceration.

CONCLUSION: Based on the findings from this study, it was concluded that *Chromolaena odorata* had an ameliorative effect on petrol-induced bronchiectasis in Wistar rats.

Keywords:

Chromolaena odorata, Petrol, Bronchiectasis, Lungs

INTRODUCTION

Petrol is a light oil that is obtained by distilling petroleum and used as a solvent and in internal combustion engines (Ezzat *et al.*, 2011). Petroleum engineers, oil or gas industry workers, fuel tanker drivers, petrol station employees, pump attendants and fuel hawkers are often exposed to petrol fumes daily for hours during the course of their work. Breathing in the fumes of petrol can occur while filling car's fuel tank, or working directly with petrol. Petrol contains organic chemicals, viz., benzene, sulphur dioxide, nitrogen oxides, pet coke, formaldehyde, silica and mercury as the active ingredients which are toxic to humans

with significant morbidity and mortality if left untreated (Rehab 2020). Previous studies have shown that repeated exposure to petrol fumes, a severe respiratory and skin irritant, cause respiratory allergy and shortness of breath in experimental animals (Okonkwo *et al.*, 2016; Iyawe *et al.*, 2012). Signs and symptoms of petrol poisoning include coughing, chest pain, wheezing, tachypnea and inflammatory reaction (Mark *et al.*, 2006).

Bronchiectasis is a disease of the bronchi that commonly involve the bronchioles, characterized by destruction of the bronchi/bronchioles, causing them to widen (Harrison, 2018; Drake *et al.*, 2010). Damaged

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bronchi/bronchioles cannot clear mucus like they're supposed to (Iyawe and Ebomoyi, 2005; Moore and Dalley, 2014). Bacteria then grow in the mucus, causing inflammation, bronchiolar mucosal and vascular ulcerations and more damage to the lungs (Mark *et al.*, 2006; Iyawe *et al.*, 2012; Okonkwo *et al.*, 2016). Bronchiectasis occurs when an irritating substance causes destruction and inflammation of the airway which then makes it difficult for oxygen to pass through the alveoli into the blood stream (Iyawe *et al.*, 2019; Mark *et al.* 2006; Ross and Wilson, 2018).

Chromolaena odorata, popularly called 'baby tea' is a traditional medicinal plant that is widely used for its wound healing property. It belongs to the family, *Asteraceae* (Buddhachat *et al.*, 2020). It is a species of flowering plant that is cultivated in Asia, China, South East Asia and the Tropics (Apori *et al.*, 2000). *Chromolaena odorata* plant has up to 6 leaves per rosette. Mature leaves are dark green, elliptical, oval and characterized by their obtuse-type apex and usually range between 30 and 35cm in length and 5 to 7cm in width ((Buddhachat *et al.*, 2020). The plant is cultivated mainly as medicinal widely in both tropical and subtropical regions of the Indian sub-continent for the treatment of cough and catarrh. Phytochemical constituents of *Chromolaena odorata* include: flavonoids, saponins, phenols, steroid, coumarine, curcubitane, triterpenoids and fatty acid (Apori *et al.*, 2000).

Literature reports that *Chromolaena odorata* leaves can be utilized in the therapy of malarial fever, allergy, asthma, chest pain, cough, catarrh and other respiratory diseases (Buddhachat *et al.*, 2020). Scientists have opined that the active principles which confer antipyretic, antihistamine, antitussive and soothing effects on the plant are the curcubitanes, triterpenoids and flavonoids. Hence, the present study investigates the effects of aqueous leaf extract of *Chromolaena odorata* on petrol-induced bronchiectasis in the respiratory system of adult Wistar rats.

MATERIALS AND METHOD

Ethical Consideration: Ethical approval was obtained from the Research Ethics Committee of the College of Medical Sciences, University of Benin, Nigeria (Approval number: CMS/REC/2012/302). Each animal procedure was carried out in accordance with approved protocols and in compliance with the recommendations for the proper management and utilization of laboratory animals used for research (Buzek and Chastel, 2010).

Plant Materials: *Chromolaena odorata* leaves were harvested from the University of Benin Farm Project, Benin City. The plant was identified at the herbarium of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

Extract Preparation: *Chromolaena odorata* leaves were oven-dried at 40°C after air-drying for 7 days. The dried leaves were

then grounded using a 2018 model mechanical grinder (Dozenmann, U.S.A). The cold maceration method was used to extract the powdered material by soaking 500g of the powdered *Chromolaena odorata* leaf in 1litre of water for 24 hours at room temperature (Apori *et al.*, 2000). The soaked *Chromolaena odorata* was filtered with the aid of cotton wool. Using evaporating dishes, the filtrate was concentrated over a hot water bath at a temperature of 60 °C to obtain 20 g concentrated jellylike extract of *Chromolaena odorata* leaf. The aqueous extraction yielded a crude extract with a yield percentage of 10.2 % (w/w), which was further purified to obtain a yield of 3.5 % (w/w) aqueous extract of *Chromolaena odorata*. The 3.5 % (w/w) aqueous leaf extract was then transferred into a sample bottle for storage in a refrigerator at 4°C.

Experimental animals: Thirty (30) adult Wistar rats of 173g-215g were purchased from the Animal House, Department of Anatomy, University of Benin, Nigeria. The animals were left to acclimatize for 2 weeks before commencement of the experiment. During this period, they were allowed access to standard animal feed and clean water *ad libitum*.

Induction of Bronchiectasis: Bronchiectasis was induced by exposing the test animals to 100 ml of petrol via a fume-distributor glass-chamber (2006 model, manufactured by Hoddler and Stoughton Group of Company, London) for 1 hour daily for 30 consecutive days (Okonkwo *et al.*, 2016). A pilot study was done on of the 30th day of the experiment which confirmed bronchiectasis.

Experimental design: In this study, 30 animals were divided into 5 groups comprising of 6 rats per-group. Animals in group A which served as control received standard feed and clean water *ad libitum*. Animals in group B were exposed to 100 ml of petrol vapor via inhalation. Animals in group C received 500mg/kg body weight per day of *Chromolaena odorata*. Animals in group D were exposed to 100 ml of petrol vapor via inhalation and received 250mg/kg body weight of *Chromolaena odorata*. Group E rats were exposed to 100 ml of petrol vapour via inhalation and received 500mg/kg body weight of *Chromolaena odorata*. The dosages were given for 30 consecutive days via an orogastric tube. The weights of the animals in each group were taken and recorded weekly and the difference noted.

Method of Sacrifice and Sample Collection: After the 30th day of exposure to petrol, the animals were weighed and then euthanized under chloroform anaesthesia and the lung of each rat was excised and fixed in 10% formal saline for 24 hours before the histological procedures. The tissues were trimmed to about 3-5 mm thick sections and processed according to the method of Drury and Wallington (2014). The trimmed tissues were histologically processed using the method of fixation, embedding and tissue staining for microscopy. Histological sections were examined under a Leica DM750 research microscope with a digital camera

(Leica ICC50) attached. Photomicrographs of the tissue sections were taken at 100x magnification.

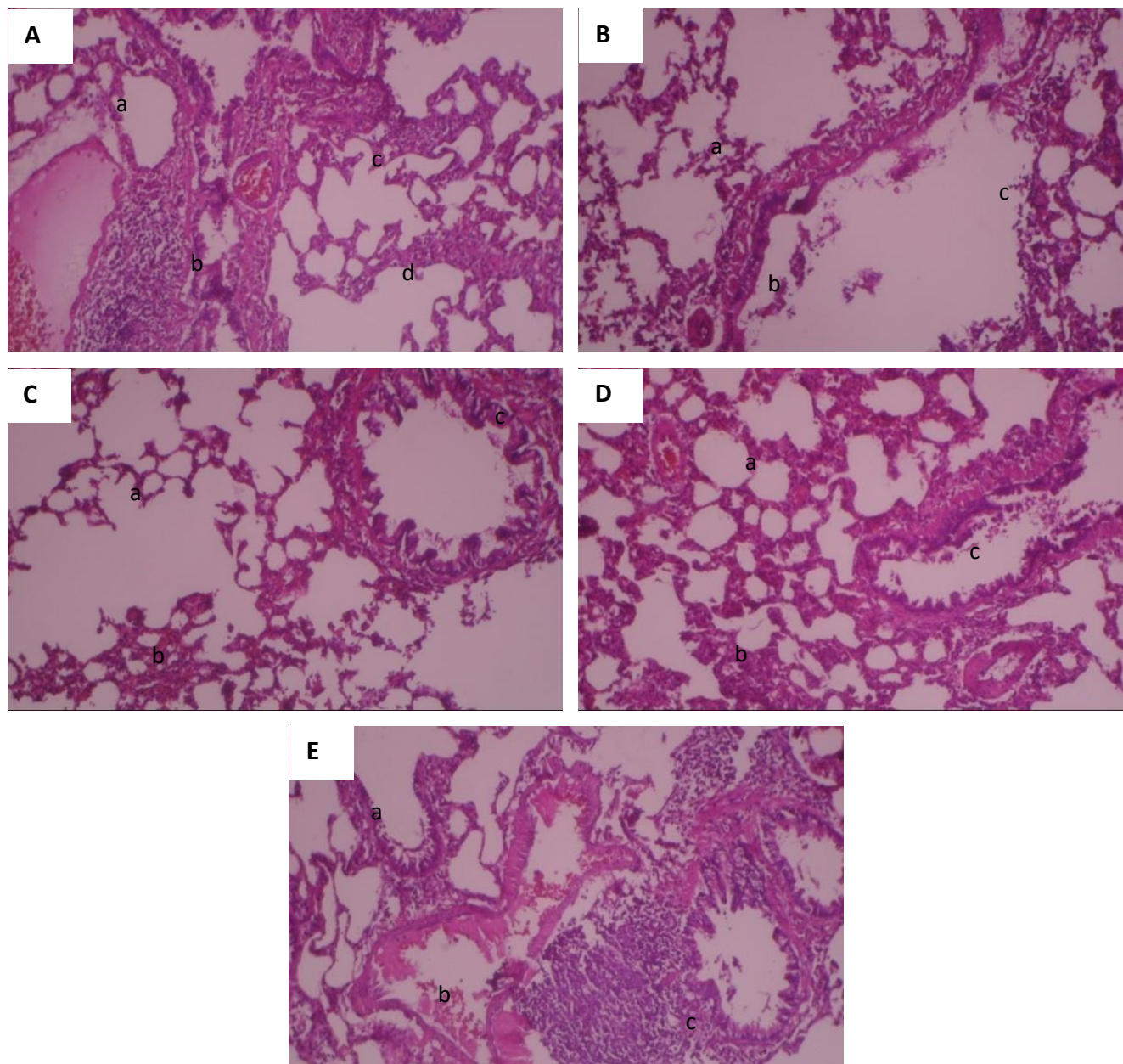


FIGURE 1: Photomicrographs of a section of lungs of the control group (A) showing normal alveolar sac (a), terminal bronchiole (b), and bronchiolar artery (c). Group B (B) exposed to 100 mls of petrol vapor only shows bronchiolar destruction (a), severe bronchiolar ulceration (b) and vascular ulceration (c). Group C (C) administered 500mg/kg body weight of *C. odorata* only shows normal architecture of alveoli (a), normal bronchiole (b) and normal vascular architecture (c). Group D (D) exposed to 100 ml of petrol vapor and 250mg/kg body weight of *C. odorata* shows normal architecture of alveoli (a), normal bronchiole (b) and normal vascular architecture (c). Group E (E) exposed to 100 ml of petrol and 500mg/kg body weight of *C. odorata* shows normal architecture of alveoli (a), mild bronchiolar mucosal ulceration (b) and focal vascular ulceration(c). (H&E; 100x).

RESULTS

The photomicrograph of the control group (group A), shows normal features of the lungs such as alveolar sacs, interstitial space, terminal bronchiole and bronchial artery (figure 1A). In the rats exposed to 100 mls of petrol (group B), there were mild bronchiolar destruction, bronchiolar ulceration and

vascular ulceration (figure 1B). The group that was given 500mg extract only (Group C), presented normal architecture of alveoli, normal bronchiole and normal vascular architecture (figure 1C). In the rats given 250mg/kg extract and exposed to 100 mls of petrol vapor (group D), there were normal vascular architecture, normal bronchiole and normal alveoli (figure 1D). The group that was given 500mg/kg body

weight extract and exposed to 100 mls of petrol vapor (group D) presented normal architecture of alveoli, mild bronchiolar mucosal ulceration and focal vascular ulceration.

DISCUSSION

Chromolaena odorata has been reported to have various medicinal uses). Literature reports that it has anti-tussive, anti-inflammatory and anti-histamine properties (Buddhachat *et al.*, 2020). Against this background, this study was conducted to evaluate the effects of aqueous leaf extract of *Chromolaena odorata* on petrol-induced bronchiectasis in adult Wistar rats.

In Figure 1 (A), the histological section of the lung of control (Group A) showed normal histoarchitecture of the lung, viz., normal alveolar sacs, terminal bronchioles, and bronchial artery while in Figures 1(B), there was destruction of the bronchioles in the lungs of the rats that were exposed to petrol alone (Group B) which occurred as a result of the body sensing a foreign body (excess accumulated petrol fumes) leading to the activation of lymphoid tissues to get rid of it.

Severe bronchiolar ulceration, vascular ulceration and destruction of the bronchioles, causing them to widen were observed which concur with previous work (Okonkwo *et al.*, 2016). These histomorphological changes indicate diseases and pathological symptoms of variety of maladies including bronchiectasis (Mark *et al.*, 2006) and this could lead to the mortality of the research animals.

As shown in Figure 1 (C) (Group C) *Chromolaena odorata* showed no negative effect on the histology of the lungs as the alveoli, bronchioles and bronchial artery were found to be histologically normal in the rats that were administered only the extract. For the rats in Group D (Figure Figure 1 (D), at low doses, *Chromolaena odorata* showed a protective effect against petrol-induced bronchiolar damage. Bronchiectasis was completely prevented and the accumulated particulate matters were cleared allowing the flow of oxygen into the alveolar sacs and release of carbon dioxide from the blood. For the rats in Group E (Figure 1(E), at high doses, *Chromolaena odorata* showed some protective effects against bronchiolar damage as the alveoli were found to be histologically normal and the bronchiolar mucosal ulceration and vascular ulceration were mild. Bronchiectasis was partially prevented which also allowed the exchange of oxygen and carbon dioxide between inspired air and blood. This shows that the high dose of *Chromolaena odorata* caused a fairly potent amelioration of the bronchiolar damage and vascular ulcerations. Our findings agree with a similar work done by Ezzat *et al.*, (2011), where they used gasoline fumes to induce pneumonia.

CONCLUSION

Chromolaena odorata had ameliorative effects against petrol-induced bronchiolar damage, bronchiolar mucosal and

vascular ulcerations. The effects were seen to be inversely proportional to the dosage as the results were more desirable at lower doses and could be compared to the control group. *Chromolaena odorata* is therefore valuable in combating bronchiectasis.

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